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Brief Report

Absence of BRAF Gene Mutation in Non-Melanoma Skin Tumors

Massimo Libra[1,†,*]
Grazia Malaponte[1,†]
Valentina Bevelacqua[1]
Roberta Siciliano[1]
Paola Castrogiovanni[1]
Alberto Fulvi[1]
Giuseppe Micali[2]
Giovanni Ligresti[3]
Maria C. Mazzarino[1]
Franca Stivala[1]
Salvatore Travali[1]
James A. McCubrey[4,5]

[1]Department of Biomedical Sciences; [2]Department of Dermatology; University of Catania; Italy

[3]Department of Experimental Oncology 2; CRO IRCCS; Aviano National Cancer Institute; Aviano, Italy

[4]Department of Microbiology and Immunology; [5]Leo W. Jenkins Cancer Center; Brody School of Medicine at East Carolina University; Greenville, North Carolina USA

[†]These authors equally contributed to this work.

*Correspondence to: Massimo Libra; Department of Biomedical Sciences; University of Catania; Italy; Tel.: +39.095.313429; Fax: +39.095.320267; Email: mlibra@unict.it

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KEY WORDS

BRAF, BCC

ABBREVIATIONS

PCR	polymerase chain reaction
BCC	basal cell carcinoma

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ABSTRACT

Basal cell carcinoma (BCC) is the most common skin cancer, and its incidence is increasing. It was proposed that the *RAS* oncogene significantly contributes to skin cancer development. Numerous *BRAF* mutations have been detected in melanoma biopsy specimens and cell lines. For the first time, in the present study, tumor biopsy specimens from 78 patients with BCC were screened for *BRAF* mutation within exons 11 and 15. Our results indicate that the *BRAF* gene does not appear to be frequently mutated in nonmelanoma skin tumors such as BCC. These data suggest that other gene alterations may cause tumor development.

INTRODUCTION

Skin neoplasms represent a heterogeneous and complex group of tumors deriving from the various structures that constitute the human skin. Basal cell carcinoma (BCC) is the most common skin cancer, and its incidence is increasing.[1] BCC is classified histologically according to generally recognized forms of growth, which can also be related to the behavior of the tumor. A simple and commonly used classification is nodular, micronodular, infiltrative and superficial.[2] There can also be mixed tumors, including a combination of any two or all of the above types.[3,4] Histological assessment remains the basis of a tumor analysis and this is especially true for BCC, where our understanding of its cellular defects remain limited. A key goal for cancer pathologists is to relate the cancer phenotype to a list of defining molecular principles.[5]

Mutation frequencies of *RAS* family genes were extensively analyzed in the early 1990s and at that time it was proposed that the *RAS* oncogene significantly contributes to skin cancer development. Now, an overall *RAS* mutation frequency of 10–20% is suggested for squamous cell carcinoma (SCC) and BCCs.[6-12] Moreover, mutation of *BRAF* has been proposed to contribute to cancer development.[13] B-RAF is a kinase that activates the RAF/MEK/ERK signal transduction cascade. Increased activity of the RAF/MEK/ERK pathway prevents apoptosis and induces cell cycle progression.[14,15] Numerous *BRAF* mutations have been detected in melanoma biopsy specimens and cell lines.[13-16] However, no previous studies have investigated whether or not *BRAF* mutation is detected in BCC. To shed light on this issue, we have analyzed a large numbers of BCC samples for *B-RAF* gene mutation.

MATERIALS AND METHODS

Cases. Tumor biopsy-specimens were isolated from 78 patients with BCC. All BCC biopsy-specimens were fixed with formalin then embedded with paraffin. The mean age (years ± SD) of cases was 68.6 ± 11.4. Forty were male and 38 were female. For the purpose of the present study, all BCC cases were reviewed and classified according to the classification recommended by the Royal College of Pathologists.[2] The site of tumor origin was the trunk in 68 cases (87%), the head and neck in ten cases (13%). The tumors were fully excised in all 78 cases.

***BRAF* mutation analysis.** All BCC biopsy specimens were screened in duplicate for *BRAF* mutation within exons 11 and 15. As control group, the DNA from two different melanoma samples harboring mutations within exons 11 and 15 were analyzed. Genomic DNA was isolated with the QIAgen Tissue Kit (Qiagen, Valencia, CA, USA). Exons 11 and 15 were amplified by polymerase chain reaction (PCR) as previously described.[16] The forward and reverse primer sequences used to amplify exon 11 were 5'-TCCCTCTCAGGCATAAGGTAA-3' and 5'-CGAACAGTGAATATT-TCCTTTGAT-3', respectively. The forward and reverse primer sequences used to amplify exon 15 were 5'-TCATAATGCTTGCTCTGATAGGA-3' and 5'-GGCCAAAAATTTAATCAGTGGA-3', respectively. DNA was denatured for 7 minutes at 94°C then subjected to 35 amplification cycles.

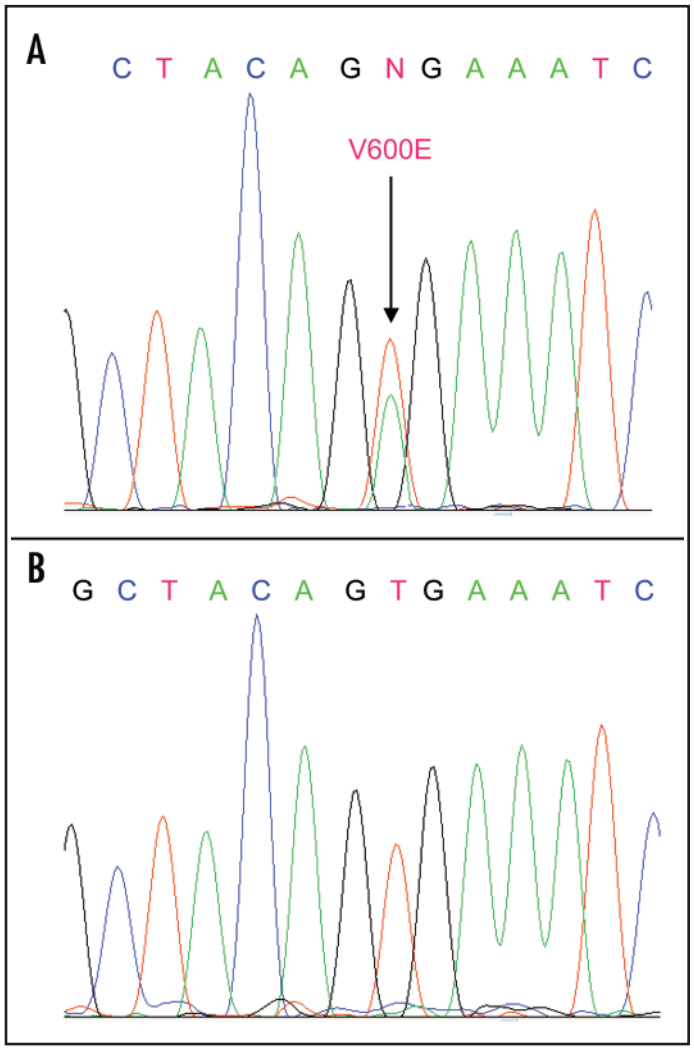

Figure 1. Mutational analysis of *BRAF* gene in basal cell carcinoma (BCC). (A) Sequence showing the *BRAF* exon 15 (V600E) mutation in a melanoma DNA (control). *BRAF* exon 15 contains the major "hot spot" found to be mutated in human cancer.[13] In all BCC samples, sequence analysis of PCR products failed to reveal mutations within both exons 11 and 15 of *BRAF* gene. (B) Example of BCC DNA wild-type for *BRAF* exon 15.

Each amplification cycle consisted of: (1) denaturation for 30 seconds at 94°C, (2) annealing for 30 seconds at 57°C, and (3) extension for 30 seconds at 68°C. DNA was incubated at 72°C for an additional 9 minutes after completion of the final amplification cycle. PCR products were separated by electrophoresis in 2% agarose then stained with ethidium bromide. Purified PCR products were sequenced with an ABI Prism 3100 Genetic Analyzer (PerkinElmer, Foster City, CA, USA) by the dye terminator protocol. The sequence of each PCR product was compared with that of GenBank accession number M95712.

RESULTS AND DISCUSSION

Previous studies have suggested that the *RAS* family genes contribute to skin cancer development.[6-12] Mutation of *BRAF* has been proposed to play a role in cancer growth, including primary and metastatic melanoma.[13-16] The identification of *BRAF* alteration in BCC may represent a new approach for both diagnosis and treatment of this non-melanoma skin tumor. Whether or not, *BRAF* mutation cooperates in BCC development has not investigated until now.

In the present study, tumor biopsy specimens from 78 patients with BCC were screened for *BRAF* mutation. In all BCC samples, sequence analysis of PCR products failed to reveal mutations within both exons 11 and 15 of *BRAF* gene (Fig. 1). Since, an interaction between the MAPK and PTEN pathways has been recently found in cutaneous melanoma,[17] we can speculate that other gene alterations, such as *PI3K* gene mutations, may cause tumor development. Moreover, an involvement of MAPK and/or PTEN pathways suggests that multikinase inhibitor agents are likely to provide important new strategies for the management of this disease. Of note, the BAY43-9006, a RAF inhibitor,[18] has been shown to inhibit also both wild-type and mutant B-RAF the growth of a human melanoma cell line carrying the *BRAF* V600E mutation.[19] Interestingly, it has been also suggested that the compound's efficacy in advanced melanoma is lower on activated mutated forms of B-RAF than on wild-type B-RAF.[19,20] In contrast, more recent studies with a new MEK inhibitor indicate that the MEK inhibitor PD0325901 may suppress growth of melanoma cells with the mutant *BRAF* gene much more effectively that cells harboring the wild-type *BRAF* gene.[21] In summary, our results are the first to indicate that the *BRAF* gene does not appear to be frequently mutated in nonmelanoma skin tumors such as BCC from patients of the Mediterranean area.

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